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VARIETY REGISTRATION

Registration of a new variety of chamomile (*Chamomilla recutita* (L.) Rauschert) CIM-Ujjwala: A novel source of acetylinic compound [(2z,8z)-matricaria acid methyl ester] for use in cosmetics and pharmaceuticals

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ABSTRACT

The plant Chamomilla recutita (Family: Asteraceae), popularly known as 'Chamomile', is a natural source of essential oil containing terpenoides and flavonoids that have high value and demand in pharmaceutical and perfumery industries. Its dried flowers are also traded owing to their extensive use in herbal tea, mouth wash and bathing products. Although this plant was introduced in India, around three centuries ago, its cultivation could not expand in the country because of lack of improved cultivars. The novel variety/chemotype CIM-Ujjwala developed during the course of this study is very promising for high flower and oil yield. The oil is also rich in acetylinic compound (2z,8z)-matricaria acid methyl ester (76-80%). This improved chemotype has the production potential of 7.00-7.50 q/ha dry flowers and 6.00-6.50kg /ha oil yield. The acetylinic compound [(2z,8z)-matricaria acid methyl ester] has been shown to be effective against hyper pigmentation of human skin and can be of tremendous use in cosmetic and pharmaceutical industries. The chemotype CIM–Ujjwala has been developed through intensive mutation breeding. This chemotype would provide high remunerative return to the growers and industries.

INTRODUCTION

The genus *Chamomilla* belonging to the family *Asteraceae* (*Compositae*), is a natural source of a commercial essential oil, containing terpenoides and flavonoids that are in high demand in pharmaceutical and perfumery industries. The flowers are also in great demand owing to their extensive uses in herbal tea and bathing products. The world demand of chamomile dried flowers are about 600 tons/year and productions is only around 300 tones. Major producers of Chamomilla oil are Germany, Slovak Republic, Italy and Bulgaria. In India its annual production is only 25 tones. Though

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this crop introduced about three centuries ago in India, its commercial cultivation could not be expended adequately due to lack of good cultivars/ varieties and germ plasm of chamomilla compled with fluctuating market demand. The germplasm of German Chamomile was thoroughly evaluated by CSIR-CIMAP and resulted in release of three improved varieties namely, Vallary, Prashant and CIMAP Sammohak that were rich in Camazulene. In course of further improvement in oil and flower yield and its other attributes like size of disc and ray florets and quality constituents of oil, the induced mutation breeding through gamma rays irradiation was initiated which ultimately led to identification of variable genotype/chemotype in respect to oil yield and oil quality attributes.

MATERIALS AND METHODS

Origin/breeding of the new strain/ variety

Under a mutation breeding programme of chamomile, the dry seeds of variety Vallary were irradiated with gamma – rays at 10- 100 kR doses in a Gamma chamber 4000A (source ^{60}Co). All the

Table 1. Overall mean values, critical variance, standard errors for pollen fertility and percent of germination in M_1 generation of chamomile

Irradiation dose (kR)	Plant height (cm)	Seed germination (%)	Plant survival (%)	Pollen fertility (%)	Range of pollen fertility (%)	C.V. (%)
10	34.50	85.60	94.60	70.50±7.70	16.50-89.00	29.40
20	30.30	84.80	92.80	59.21±8.50	10.00-89.00	27.20
30	25.50	81.00	88.00	70.00±2.58	26.80-88.50	30.86
40	22.20	80.00	84.60	60.90±4.50	15.70-86.00	40.10
50	20.30	77.50	84.00	58.60±4.80	6.53-91.00	50.55
60	18.10	76.39	83.80	58.24±3.10	3.00-78.00	56.10
70	15.80	69.00	80.00	50.00±3.75	7.00-75.00	30.28
80	12.70	64.00	58.00	48.37±2.00	3.50-83.00	40.13
90	10.50	50.00	40.00	10.23±3.60	4.00-69.00	56.10
100	8.60	40.00	30.50	5.00±2.00	0.00-10.00	40.20
Control (0 kR)	35.00	90.50	97.50	91.50±5.20	90.00-94.70	8.70

C.V. – Critical variance

Table 2. Average performances of individual plants/genotypes for different yield component traits of chamomile genotypes in M1

Entries	Days to flower (50%)	Plant height (cm)	Branches/plant	Spread (cm^2)	Dry flower yield/Plant (gm)	Oil content (%)
SELM-1	49.11±2.35	34.44±2.88	12.11±3.33	2834.89±412.84	90.74±6.00	1.06±2.25
SELM 20-12-15	52.72±4.50	26.19±2.80	11.00±5.50	3098.81±215.23	110.23±2.10	0.77±1.50
SELM 70-3-30	47.40±2.88	25.70±1.76	11.20±1.86	2010.60±325.29	120.56±3.00	0.80±2.10
M-20-15-2	43.00±4.00	27.16±3.50	9.33±5.00	2279.25±254.12	130.58±6.00	0.50±1.60
M-30-25	40.80±5.80	30.18±4.50	8.27±4.78	2029.91±258.36	90.12±5.89	0.56±2.50
M-30-1	41.99±4.70	31.10±3.60	9.36±5.96	2700.40±330.12	130.63±2.56	0.62±3.00
M70-1-22	41.00±2.00	28.00±4.07	6.00±0.92	2448.00±415.55	80.89±5.03	0.56±1.23
YEL-1	49.11±2.35	34.44±2.88	12.11±3.33	2834.89±412.84	90.74±6.00	1.06±2.25
24-20	40.50±3.50	36.50±3.55	10.75±8.79	3816.50±214.98	130.50±2.56	0.77±3.20
CIM Sammohak	42.90±5.41	37.70±2.56	7.30±7.45	2204.80±268.78	65.65±4.50	0.62±1.35
Vallary	49.70±6.20	30.20±5.46	10.50±6.89	2821.10±418.23	120.59±3.50	0.50±1.50
Prashant	42.60±4.25	24.40±1.53	6.60±0.51	1609.60±299.03	70.00±2.00	0.50±1.40
$t_{10\ df}$	34.898	22.425	15.217	13.755	13.772	12.559
Mean	44.7018	30.1427	9.3109	2532.1691	103.6809	.6600
SD	4.24832	4.45807	2.02935	610.54613	24.96865	.17430
S.E. mean	1.28092	1.34416	.61187	184.08658	7.52833	.05255

Table 3. Mean performance of the seven selected mutants in M2 generations

Entries	Oil comtent	Oil colours	Chamazulene (%)	Bisabolol oxide A (%)	Bisabolol oxide B (%)	α–Bisabolol (%)	Bisabolone oxide (%)	(2*Z*,8*Z*)-Matricaria acid methyl ester (%)	2e, 8z matricaria ester (%)
SELM-1	0.90	Light brown	-	-	-	-	-	80.50	6.85
SEIM20-12-15	0.70	Light blue	1.23	19.74	9.31	6.08	10.93	-	-
SELM70-3-30	0.66	Light green	0.40	12.00	12.04	5.07	11.33	-	-
M-20-15-2	0.60	green	0.66	32.41	10.47	7.48	7.63	-	-
M-30-25	0.65	Dull white	0.36	31.46	10.30	6.89	7.51	-	-
M-30-1	0.56	Light green	0.54	19.73	9.26	9.15	8.12	-	-
M70-1-22	0.65	Blue	0.64	18.41	9.26	9.15	8.12	-	-
CIMAP Sammohak	0.75	Very dark blue	12.98	20.12	11.01	9.15	11.33	-	-
Vallary		Dark blue	7.23	19.74	9.31	6.08	-	-	-

treated sets of seeds along with those of the control variety Vallary were planted under homogenous field conditions at the Institute's experimental farm within one hour of irradiation.

Visual observations were recorded on M_1 plants progeny for plant vigour and the number and size of the flowers. No significant difference among them was noted (Table 1 & 2). Seeds were subsequently collected separately from all normal looking healthy plants and raised in widely spaced (50 cm × 50 cm) plant-to-progeny rows. Thus 29 M_2 were advanced further in M_3 generation after exercising within and between family selection and morph metric data were gathered on them. Once again within and between family selections were applied in M_3 generation and only 7 variants emerging from families of 10, 20, 40 and 70 kR doses were advanced to M_4 generation for further evaluation (Table 3). All the variants were carried up to M_{12} generation to wipeout the possibility of segregation. They were almost stabilized in M_{12}. Finally, three superior mutants 'SELM-1', 'SELM20-12-15',' SELM70-3-30' and two checks variety Vallary and CIM-Sammohak were evaluated in an Initial Evaluation Trial (IET- in the year 2013-2014) in paired rows of 3 m each, plot size 1.5 m^2 (Table 4). Again all the 5 (3+2 checks)entries were evaluated in a Bench Scale Trial (BST 2014-15 and 2015-16) in a randomized block design repeated three times in plots of 3 m x 2 m = 6m^2. These three best-performing mutants for flower and oil yield of best quality were placed in pilot scale trial (100 m^2 plot size 2016-2017) along with the two controls.

The elite Mutant SELM–1 maintained its superiority over all the checks for flowers and oil yield of superior quality. The elite mutant line named as variety CIM- Ujjwala and was released for commercial cultivation.

Table 4. Performance of the selected mutants for most economic traits of chamomile

Entries	IET (3m paired rows, RBD, 3 reps)			BST (3x2m = 6m, RBD, 3 reps.)			PST (100 m^2)				
	Dry flower yield (kg/plot)	Oil content (%)	Oil yield* (ml/ plot)	Dry flower yield (kg/plot)	Oil content (%)	Oil yield (ml/plot)*	Dry flower yield (kg/plot)	Oil content (%)	Oil yield (ml/plot)	Dry flower yield (ql/ha)	Oil yield* (kg/ha)
SELM-1	0.437	0.92	3.982	0.502	0.883	4.449	6.90	0.85	58.65	6.90	5.87
SEIM20-12-15	0.260	0.61	1.529	0.386	0.61	2.370	4.75	0.65	30.88	4.75	3.09
SELM70-3-30	0.215	0.56	1.208	0.313	0.623	1.373	4.65	0.60	27.90	4.65	2.79
Vallary	0.282	0.64	1.828	0.351	0.67	2.340	4.57	0.57	26.049	4.57	2.61
CIMAP Sammohak	0.342	0.81	2.783	0.362	0.77	2.773	5.98	0.75	44.85	5.98	4.49
CD (5%)	0.0576	0.209	0.614	0.0627	0.0781	1.208					
CD (1%)	0.0826	0.298	0.877	0.0895	0.112	1.726					

*- Essential oil yield potential; IET-Initial Evaluation Trial: BST- Bench Scale Trial; PST- Pilot Scale Trial

Gas chromatographic analysis

Varian, CP-3800 GC fitted with TG-WAXMS column (30 m x 0.32 mm i.d., film thickness 0.25 µm) was used to identify and quantify the essential oil components of all the essential oils. Hydrogen was used as carrier gas at constant pressure of 7 psi and worked in split mode with split ratio of 1:40. The column oven was programmed from 40°C to 80°C (hold for 9 min) at the rate of 3°C/min and then programmed to 80°C to 120°C at the rate of 2°C/min and finally 120°C to 250°C at the rate of 5°C/min injector and detector (FID) were maintained at 250°C respectively**.** For GC-MS, TG-WAXMS fused silica capillary column (30 m x0.32 mm i.d., film thickness 0.25 µm) was used in a PerkinElmer Clarus® 680 GC interfaced with a Clarus® SQ 8C Quadrupole mass spectrometer. Programmed Split/Splitless (PSS) injector temperature was 250°C with a split ratio of 1:40. Column oven was programmed from 40°C to 120°C (hold for 9 min) at the rate of 3°C/min and increased to 120°C to 140°C (hold for 2 min) at the rate of 2°C/min and finally programmed to 140°C to 250°C (hold for 2 min) at the rate of 5°C/min. Helium was used as carrier gas at constant flow of 1.7mL/min. Transfer line and source temperature were 200°C; electron impact ionization mode was used with ionization energy of 70eV; scan duration comprised of scan time (0.5 sec) and inter- scan delay (0.15 sec) and mass scan range 40-450 amu.

Characterization was achieved on the basis of retention time, elution order, relative retention index using a homologous series on *n*-alkanes (C_7-C_{30} hydrocarbons, Polyscience Corp. Niles IL), co-injection with standards in GC-FID and GC-MS (Aldrich and Fluka), mass spectral library search NIST/EPA/NIH version 2.0 g and Wiley Registry of Mass Spectral Data (9thediion) and by comparing with mass spectral literature data.

NMR experiment

A Bruker Avance 500 MHz instrument was utilized for ^{1}H (500 MHz), ^{13}C NMR (125 MHz), DEPT, COSY, HSQC, HMBC experiments using Tetramethylsilane (TMS) as an internal standard. About 30 mg (2*Z*,8*Z*)-Matricaria ester were dissolved in $CDCl_3$ and spectral data were recorded. Chemical shifts are reported in ppm units (multicipility; s-singlet; d-doublet; q-quarter; m-multiplet; br-broad).

Table 5. Composition of the essential oil of flower head of *Chamomile*.

Constituents'	RI_a	RI_b	Istweek	2nd week	average	Identification method
(E)-β-farnesene	1660	1664	1.68	0.76	1.22	RT, MS
Germacrene D	1696	1707	nd	0.13	0.13	RT, MS
Octanoic acid	2037	2043	0.69	0.89	0.79	RT, MS
(2*E*)-Lachnophyllum ester (1)	**2114**	**2114**	**0.05**	**0.13**	**0.09**	RT, MS
(2*E*,8*Z*)-Matricaria ester (2)	**2169**	**2181**	**4.94**	**7.67**	**6.30**	RT, MS
Unidentified (M^+ 176)	2213	2217	0.42	0.43	0.42	RT, MS
(8*Z*),2,3-Dihydromatricaria ester (3)	**2218**	**2222**	**1.35**	**1.95**	**1.65**	RT, MS
Unidentified (M^+ 176)	2229	2233	0.15	0.25	0.20	RT, MS
(2*Z*)-Lachnophyllum ester (4)	**2238**	**2240**	**3.65**	**3.81**	**3.73**	RT, MS
(2*E*,8*E*)-Matricaria ester (5)	**2245**	**2259**	**1.35**	**1.55**	**1.45**	RT, MS
Tricosane	2295	2300	0.19	0.30	0.24	RT, MS
(2*Z*,8*Z*)- Matricaria ester (6)	**2304**	**2313**	**80.6**	**76.7**	**78.7**	RT, MS, ^{1}H-NMR
(2*Z*,8*E*)- Matricaria ester (7)	**2396**	**2405**	**0.98**	**0.80**	**0.89**	RT, MS,
(2*E*)-Dihydromatricaria ester (8)	**2418**	**2436**	**0.09**	**0.08**	**0.08**	RT, MS
Pentacosane	2500	2500	0.12	nd	0.12	RT, MS
(5*E*,9*Z*)-Matricaria lactone	2570	2578	0.33	0.33	0.33	RT, MS
Total			96.6	95.8	--	

RI_a: Calculated retention Indices: TG-WAXMS column (30 m x 0.32 mm i.d., film thickness 0.25 µm); RI_b: Reported retention indices (Planta Medica, 1996 (62), 329-332); nd: not detected.

Table 6. Relative FID% of the essential oil of II[nd]week collection of *Chamomile* in two consecutive days of distillation.

Constituents,	RI_a	RI_b	Day 1	Day 2	average	identification method
β-Caryophyllene	1601	1596	nd	0.07	0.07	RT, MS
(E)-β-farnesene	1660	1664	0.76	0.23	0.49	RT, MS
Germacrene D	1696	1707	0.13	0.11	0.12	RT, MS
Octanoic acid	2037	2043	0.89	0.91	0.90	RT, MS
(2*E*)-Lachnophyllum ester	2114	2114	0.13	0.06	0.09	RT, MS
(2*E*,8*Z*)-Matricaria ester	2169	2181	7.67	4.87	6.27	RT, MS
Unidentified (M^+ 176)	2213	2217	0.43	0.08	0.25	RT, MS
(8*Z*),2,3-Dihydromatricaria ester	2218	2222	1.95	1.55	1.75	RT, MS
Unidentified (M^+ 176)	2229	2233	0.25	0.07	0.16	RT, MS
(2*Z*)-Lachnophyllum ester	2238	2240	3.81	4.81	4.31	RT, MS
(2*E*,8*E*)-Matricaria ester	2245	2259	1.55	1.10	1.32	RT, MS
Tricosane	2295	2300	0.30	0.18	0.24	RT, MS
(2*Z*,8*Z*)-Matricaria ester	2304	2313	76.72	78.50	77.61	RT, MS, ^{1}H-NMR
(2*Z*,8*E*)-Matricaria ester	2396	2405	0.80	1.07	0.93	RT, MS
(2*E*)-Dihydromatricaria ester	2418	2436	0.08	0.08	0.08	RT, MS
Pentacosane	2500	2500	nd	0.15	0.15	RT, MS
(5*E*,9*Z*)-Matricaria lactone	2570	2578	0.33	0.28	0.30	RT, MS
Total			95.8	94.12		

RI_a: Calculated retention Indices: TG-WAXMS column (30 m x 0.32 mm i.d., film thickness 0.25 µm); RI_b: Reported retention indices (Planta Medica, 1996 (62), 329-332); nd: not detected.

RESULTS AND DISCUSSION

Essential oil of chamomile flower head was extracted by hydrodistillation in Clevenger type apparatus. GC-FID and GC/MS analysis of the essential oils led to the identification of fourteen constituents, comprising 95.8 to 96.6% of the total essential oil compositions with polyacetylenes (93.34%) as the most exclusive class (Table 5) followed by sesquiterpene hydrocarbon (1.68%). The most striking feature of the flower oil is that it contained high proportion of polyacetylenes with (2*Z*,8*Z*)-matricaria ester being the most dominant compound (78.7%). Interestingly, (2*Z*,8*Z*)-matricaria ester (75.23%) rich composition from *M. perforata* had previously been reported from Germany[1]. Till date, there has been no report on matricaria ester rich essential oil of *Matricaria* spp. from India. Other polyacetylene derivatives have also been identified. Interestingly, *(2Z,8Z)*-matricaria ester was found in higher amount on 1[st]collection of first week (80.6%) while 2[nd] week of collection contain 76.7%. Moreover, the 2[nd] week collection upon distillation continued on day two led to the oil recovery (Table 6).

The proportion of (2*Z*,8*E*)-matricaria ester decreases from 1[st] week to 2[nd] weeks collection but 2[nd] week redistilled material showed an increases up to 1%. In a similar way (2*E*,8*E*)-matricaria ester also increases from 1.35% to 1.55% in between 1[st] and 2[nd] week and decrease in re-distillation of 2[nd] week (Table 6). The presence of β-caryophyllene was observed in oil collected on day 1, instead, small percentage (0.07%) on day 2 distillation was recorded. Another isomer of polyacetylene showed major variation on both days of distillation. (2*Z*)-lachnophyllum ester has shown only 0.16% of difference in 1[st] and 2[nd] week but it gradually increases up to 1.0% upon redistillation of second week sample on day 2.

NMR Characterization of (2*Z*,8*Z*)-Matricaria ester

MS m/z (%BPI): 174 [M^+] (100), 103 (60.9), 159 (59.1), 77 (36.4), 75 (26.1), 143 (25.9), 63 (24.5), 115 (23.6), 89 (22.1), 131 (21.9).^{13}C NMR (125 MHz, $CDCl_3$): δ164.77(C-1), 130.66 (C-2), 122.19 (C-3), 85.74 (C-4), 83.62 (C-5), 77.84 (C-6), 77.78 (C-7), 51.66 (-OCH_3), 16.63 (-CH_3).

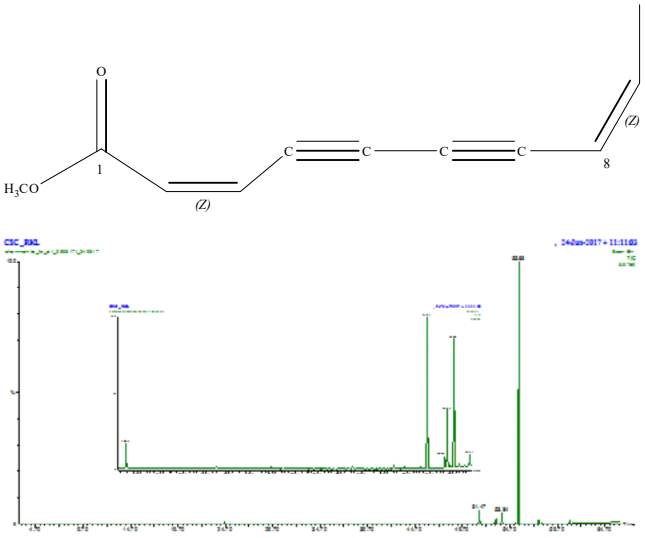

Figure 1: CIM-Ujjwala: Chromatogram of Essential Oil

Figure 2: Field view of variety CIM- Ujjwala

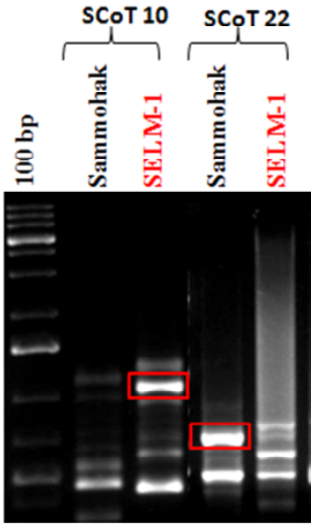

Figure 3: Variety CIM Ujjwala (SELM-1) and CIM Sammohak scot marker

Table 8. Distinct morphological features and other features

Characters	CIM- Ujjwala
Plant height (cm)	60
Date of flowering (50%)	100-120 days
Growth habit	Semi close
Leaf colour	Dark green
Leaf size	Very long and thin
Primary branches/plant	10
Dry flower yield (q/ha)	7.00-7.50
Oil content (%)	0.88
Oil colour	Light brown
Oil yield (kg/ha)	6.00-6.50
(2Z,8Z)-Matricaria acid methyl ester (%)	76-80.00 %
Other features	Late maturity

Table 7. Variation in essential oil constituents in different oil colors

Sl. No.	Entries	Oil colours	Chamazulene (%)	Bisabolol oxide A (%)	Bisabolol oxide B (%)	α–Bisabolol (%)	Bisabolone oxide (%)	(2Z,8Z)-Matricaria acid methyl ester (%)	2e,8z matricaria ester (%)
1.	SELM-1	Light brown	-	-	-	-	-	**76.70**	**7.77**
2.	SEIM20-12-15	Light blue	1.23	9.70	3.38	3.05	10.93	-	-
3.	SELM70-3-30	Light green	0.40	12.00	12.04	5.07	11.33	-	-
4.	Vallary	Blue	7.23	19.74	9.31	6.08	-	-	-
5.	CIMAP Sammohak	Very dark blue	12.98	20.12	11.01	9.15	11.33	-	-

Statement of distinction/ Breeder's claim: Variety CIM- Ujjwala is a tall (60cm) genotype with dark green long and thin distinct leaves in colour with the thick stem. This strain is bearing very big flower in size including ray and disc floret.

The strain has the following DUS (distinctiveness, uniformity and stability) characteristics.

1. The strain is morphologically distinct from other Chamomile varieties and clearly identifiable by

its very big flower in size including ray and disc florets.

2. It has dark green long and thin leaves with thick and stout stem.
3. Essential oil extracted from this strain contain higher amount of acetylinic compound [(2z, 8z)-matricaria acid methyl ester (76-80% (Table 7))
4. Strain is also lodging resistant.

The other distinguishing morphological features of this variety are given in Table 8.

Statement of distinction: The Variety CIM-Ujjwala is a tall stretcher with green stem, the high and big number of flowers/plant, as the distinct morphological features:

The variety CIM-Ujjwala was released for commercial cultivation in the year 2018. About 1.00 Kg seed of this variety will be available in the month of April.

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